

Gametophyte of the Andean Fern *Polystichum pycnolepis* (Kunze ex Klotzsch) T. Moore (Dryopteridaceae)

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ABSTRACT.—The gametophytic generation of *Polystichum pycnolepis* (Dryopteridaceae), including spore germination, morphological development of the gametophytes, major vegetative features and sexual expression, was studied. Spore germination was of the *Vittaria* model and the developmental pattern was of the *Aspidium* model. Adult gametophytes were cordate and hairy, with unicellular hairs located in the margins and the surface of the prothalli. In addition, hairs of *P. pycnolepis* were papillate, secretory and the marginal ones septate. Gametangia were of the normal type described for leptosporangiate ferns. The gametophytes produced at first instance a high proportion of female gametophytes. *Polystichum pycnolepis* formed few bisexual gametophytes with scarce antheridia. No male gametophytes for this species were detected. Thus, the species seemed to be promoting intragametophytic selfing and, to a lesser extent, intergametophytic unions between bisexuals and females.

KEY WORDS.—gametophyte, development, reproduction, Andes, Peru, *Polystichum pycnolepis*

Polystichum Roth. (Dryopteridaceae) is a large genus, with more than 250 species, worldwide in distribution (Tryon and Tryon, 1982). It is rare or absent in lowland tropical areas and best represented in the mountains of tropical and warm-temperate climates (Kramer, 1990) with a great diversity in eastern Asia (Lu *et al.*, 2007). Around 70 species are present in the neotropics. *Polystichum pycnolepis* (Kunze ex Klotzsch) T. Moore is a terrestrial montane plant that occurs in Colombia, Venezuela, Ecuador, Peru and Bolivia. Despite the diversity of species of *Polystichum*, not much research has been done on its gametophytic phase (but see Paterson, 1942; Nayar *et al.*, 1968; Nayar and Kaur, 1969; Chandra and Nayar, 1970; Sato and Saki, 1980; Greer and McCarthy, 1997; Muccifora and Gori, 1997; Pangua *et al.*, 2003; Nazario and Arreguín, 2007), and much less with the neotropical species.

The aim of this work was to study spore germination, the prothallial development, the morphology of the adult gametophyte and the sexual behavior of *P. pycnolepis*.

MATERIAL AND METHODS

Spores for cultures were taken from sporophytes collected in Peru, Cuzco Department, Urubamba Province. The following are the locations of the

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collections: de Ollantaytambo hacia el Puerto de Málaga, 13°10'36.9"S 72°17'19.4"W, 3.440 m, ribera umbrosa de un arroyo, *Gabriel y Galán s/n*, 29/04/2008. The material was identified following Tryon and Stolze (1991). Vouchers are deposited in MA.

Each spore sample for cultures were taken from separate sporophytes kept dry at room temperature for around 8 months. Gametophytes were grown under fluorescent light on a 12-h light, 12-h dark cycle at $20 \pm 2^\circ\text{C}$, in 6 cm plastic Petri dishes. Multispore cultures on mineral agar medium (Dyer, 1979) were established by sacking fertile pinnae on a weigh paper, and placing the obtained spores in the Petri dishes. The sowing was replicated twice for each sample. Percentage of germination was recorded on every third day for a random sample of 50 spores from each of the two plates, until the maximum germination was reached. Spore sizes given are the mean of 30 measurements. To study the stages of gametophyte development, random samples were taken weekly, from the beginning of spore germination until sexual maturity. Gametophytes were stained with chloral hydrate acetocarmine (Edwards and Miller, 1972), mounted in water and observed under a light microscope. Some *in vivo* observations were also made.

RESULTS

The spores of *P. pycnolepis* were monolete, ellipsoidal, $48.0 \times 34.5 \mu\text{m}$ in equatorial view, with an irregular cristate perispore. The first sign of germination was detected after 7 days from sowing, when some spores (ca. 5%) showed a rhizoid; a few days later, the first prothallial cell appeared, growing perpendicular to the rhizoid. Germination percentage was relatively low, as the maximum percentage achieved was of ca. 30%, around 21 days after sowing. Figure 1 shows the progress of the germination percentages over the days considered.

In *P. pycnolepis*, the prothallial cell emerged from the spore and divided to form a short filament of 3–5 cells with a length of 90–120 μm (Fig. 2A). At this stage, ca. 7 days after germination, the terminal cell of the filament developed an apical hair that limits its growth. The apical cell divided longitudinally, so the hair was finally located in one of the daughter cells, which became somewhat quiescent. Further divisions of the apical daughter cell without hair and by the subapical cells composed the planar plate (Fig. 2B). For this lop sided divisions, the bidimensional plate was somewhat asymmetric in the apical region, but soon, ca. 30 days after germination, this asymmetric condition was lost and a pre-cordate symmetric shape was reached (Fig. 2C). Hairs were continuously formed while the meristem developed a clear notch and the wings (Fig. 2D). Mature pre-sexual gametophytes arose ca. 60 days after germination, and were of the cordate hairy type, of about 4 mm in width, with ample, symmetric, well-developed wings (Fig. 2E).

The gametophytes of *P. pycnolepis* began to form marginal unicellular capitate hairs (Figures 2C–E, 3A), of about 30–40 μm in length, after 20 days of germination. Superficial hairs developed after ca. 50 days of germination,

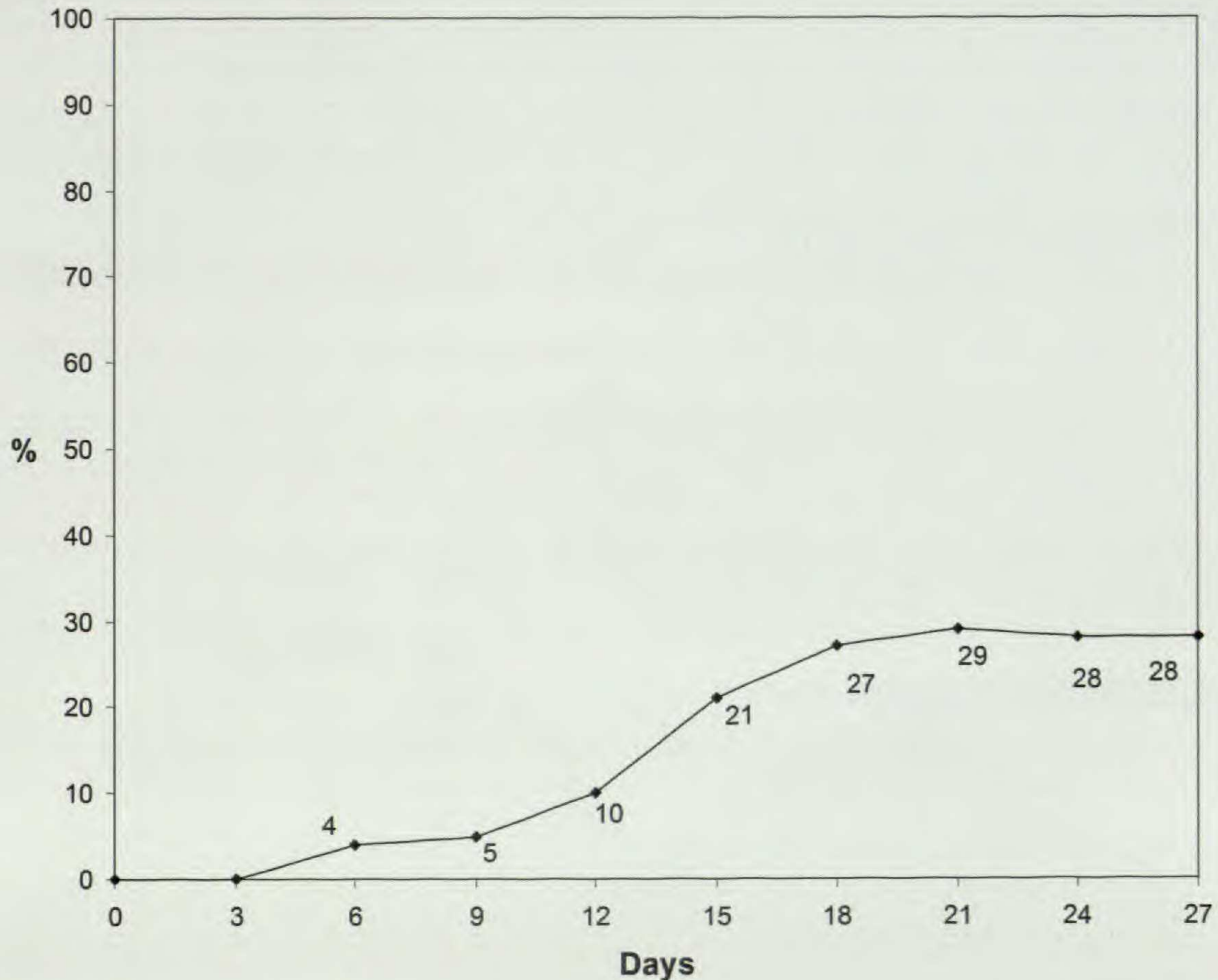


FIG. 1. Changes in the germination percentages of *Polystichum pycnolepis*, over 27 days.

achieving its maximum density during the gametangia formation around the archegonia in the ventral surface. These superficial hairs were also unicellular and capitate, but longer than the marginal ones, of about 50–60 μm . When the prothalli reached the adult stage, most of the marginal hairs became secretors, accumulating an apical coat of extracellular secretion (Fig. 3A). In this adult stage, the apical region of both the marginal and the superficial hairs changed to become papillate. At the same time, the base of the marginal hairs formed a somewhat undulate septum (Fig. 3B).

About 60–70 days after germination, gametangia were observed in some prothalli of *P. pycnolepis*. Antheridia formed in small numbers and on some prothalli just one antheridium was observed (Fig. 3C), while archegonia were abundant (Fig. 3D). From the beginning of the sexual stage until the end of the observation period (ca. 250 days from germination), female and rarely bisexual prothalli were formed, and no male gametophytes were observed.

DISCUSSION

The size and ornamentation of the spores of *P. pycnolepis* fall within the ranges of variation and types previously reported for the genus (Tryon and Lugardon, 1990). The spore germination is of the *Vittaria* type (Nayar and

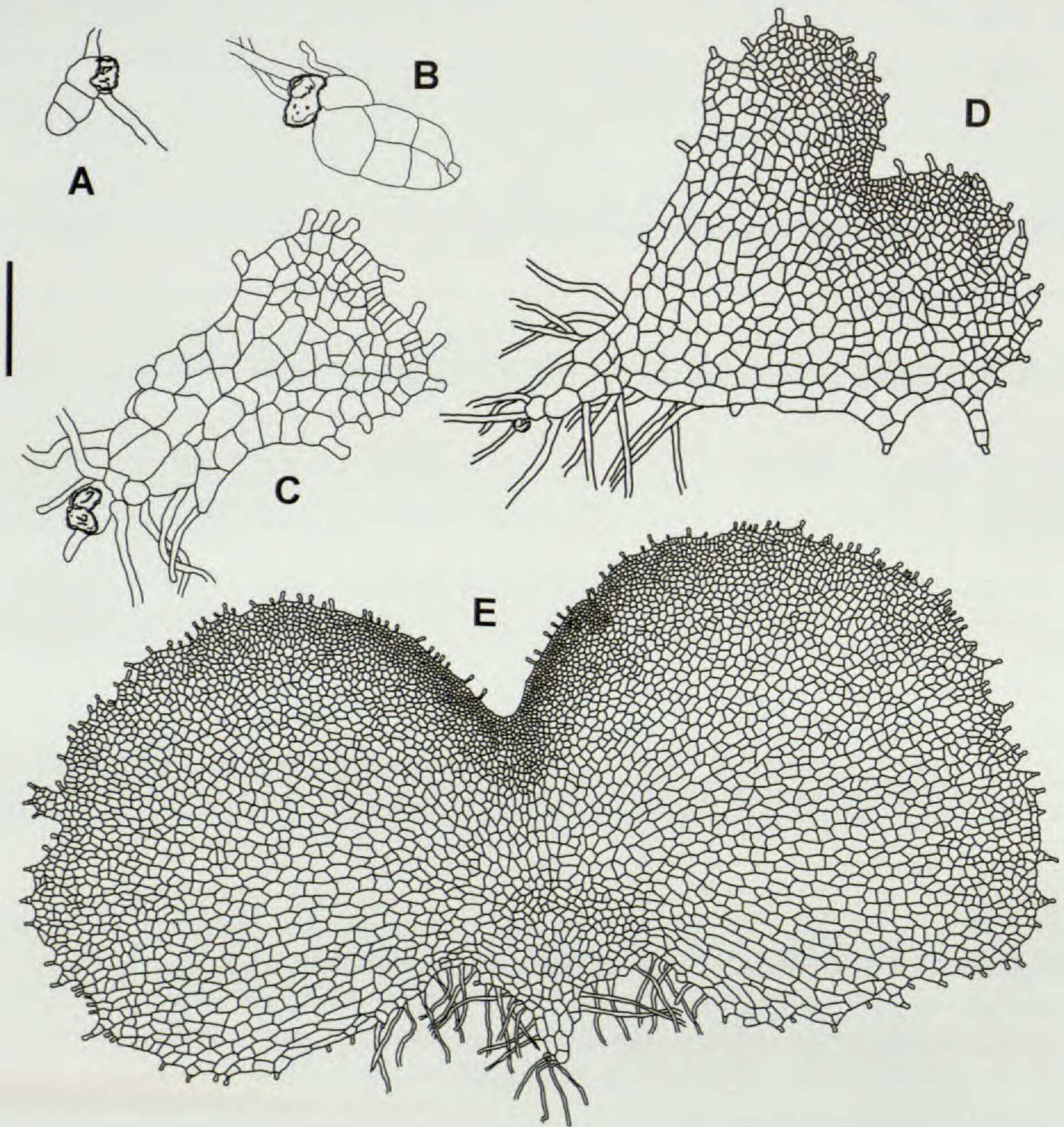


FIG. 2. Gametophyte development of *Polystichum pycnolepis*. A: filament stage, 7 days. B: initial planar stage, 15 days. C: planar non cordate gametophyte, prior to cordate stage, 30 days. D: cordate immature stage, 40 days. E: adult prothallus, 60 days. Days are measured from the germination. Bar= 90 μm in A, B; 160 μm in C; 350 μm in D; 500 μm in E.

Kaur, 1968), which has been reported on other *Polystichum* species (Chandra and Nayar, 1970; Nazario and Arreguín, 2007).

The prothallial development found in *P. pycnolepis* is of the *Aspidium* type (Nayar and Kaur, 1969), in which the germination filament ends in a hair. This kind of development is usual in the Dryopteridaceae (Nayar and Kaur, 1969), and it has been reported in many of the principal genera (Mendoza *et al.*, 1999; 2002), including *Polystichum* (Chandra and Nayar, 1970; Atkinson, 1973). Over the general *Aspidium* type described by Nayar and Kaur (1969), several

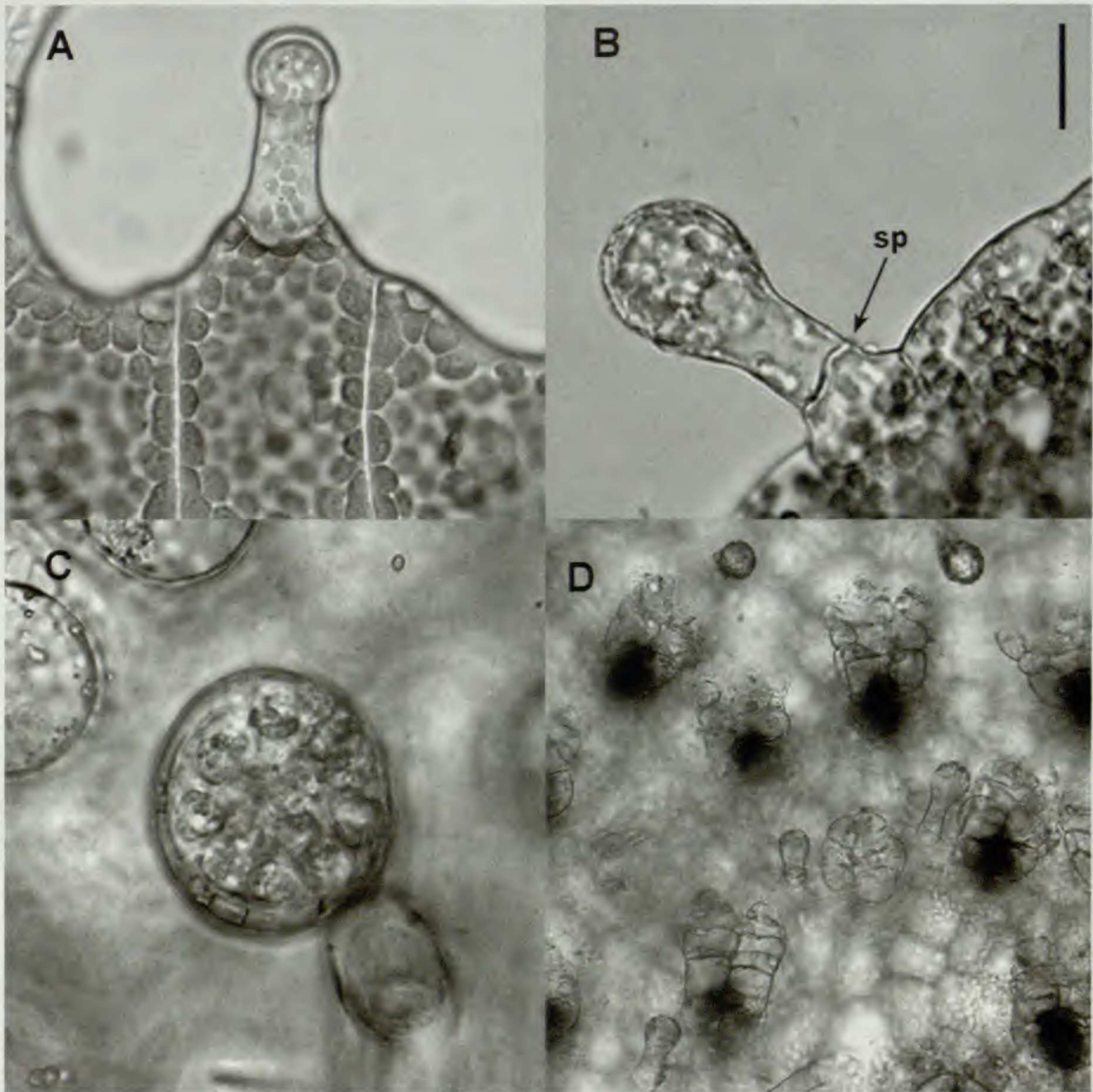


FIG. 3. Vegetative and reproductive features of *Polystichum pycnolepis*. A: marginal unicellular hair on an adult pre-sexual prothallus, 60 days. B: marginal septate papillate hair, in an old sexual prothallus, 180 days; sp=septum. C: antheridium, 180 days. D: archegonia, 180 days. Days are measured from the germination. Bar = 20 μm in A; 15 μm in B, C; 50 μm in D.

variations have been reported. *Polystichum pycnolepis* deviated from the general model in the formation of a longitudinal division of the apical cell, after the formation of the apical hair, so this is located in one of the daughter cells. This fact has been also found in other *Polystichum* species, for example in *P. biaristatum* (Blume) T. Moore., while others, as *P. auriculatum* (L.) C. Presl, do not divide the apical cell (Nayar and Kaur, 1969).

Unicellular papillate secretory hairs as those of *P. pycnolepis* have been found in several Dryopteridaceae taxa, as in other *Polystichum* species (Chandra and Nayar, 1970; Nayar and Kaur, 1971; Nazario and Arreguín, 2007), *Dryopteris* (Pérez García *et al.*, 1999), *Olfersia* (Mendoza *et al.*, 2002)

and *Arachniodes* (Chandra and Nayar, 1970; Mendoza *et al.*, 1999). In these species, hairs are non septate or this character is unknown, while basally septate hairs, as those found in *P. pycnolepis*, have been reported from genus of related families, as *Cyclopeltis* (Stokey, 1960). In some other *Polystichum* species marginal non secretory swollen hairs and papillate secretory hairs have also been found mixed (Nayar and Kaur, 1971). In *P. pycnolepis* the superficial hairs are longer than the marginal ones, a pattern that seem to be normal (Nayar and Kaur, 1971).

The sex organs in *P. pycnolepis* are typical for leptosporangiate ferns (Nayar and Kaur, 1971). The rare formation of antheridia in *P. pycnolepis* can be interpreted in various ways. Unfavorable conditions tend to increase the maleness of prothalli whereas extremely favorable conditions, as artificial cultures, could cause in the formation of strictly archegoniate prothalli (Nayar and Kaur, 1971). In a more detailed study, some *Polystichum* species, as *P. ordinatum* (Kunze) Liebm., have been reported to form the male gametangia at an advanced age, around 366 days after germination (Nazario and Arreguín, 2007). But in this case, also archegonia were formed later (218 days) than in *P. pycnolepis* (70 days). The observation in *P. pycnolepis* of antheridia coexisting with archegonia in an early sexual stage of development implies a very different strategy than the observed in *P. ordinatum*; in the latter, intergametophytic crossing seems to be promoted, as only unisexual prothalli have been detected. Other authors have mentioned the influence of light in the development of gametangia, in the sense that an intense light source could inhibit the formation of antheridia (Paterson, 1942; Raghavan, 1989). In some of these experiments, apogamous *Polystichum* species were involved, as *P. tsus-simense* (Hook.) J. Sm. Apogamy is known to be present in the genus, but that is not the case of *P. pycnolepis*; again, the reproductive strategy in *P. pycnolepis* is very different than that of *P. tsus-simense*. Some other studies (Pangua *et al.*, 2003) with the European species *P. aculeatum* (L.) Roth ex Mert. and *P. lonchitis* (L.) Roth, reported the presence of only bisexual prothalli, while *P. setiferum* (Forssk.) Moore ex Woy. had both female and bisexual prothalli. This latter reproductive strategy, despite the limited presence of antheridia, is likely to be happening in *P. pycnolepis*, which would be favoring both intergametophytic and intragametophytic crossings. Nevertheless, both *P. aculeatum* and *P. setiferum* had an antheridiogen system, which is not operating in *P. pycnolepis*.

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